NANOFLUIDIC LAB-ON-A-CHIP FOR BIOMOLECULE DETECTION

Interim Report

JPL Task 1010

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A. OBJECTIVES

We proposed to develop a nanofluidic lab-on-a-chip chromatographic system that may potentially provide detailed information on the geochemical and biological history of soil, ice, and water samples by identifying and quantifying a broad range of polar and non-polar organic compounds. This study has followed three task areas identified in the first year task plan: 1) design and fabrication of a nanofluidic chromatographic column based on the principles of size exclusion chromatography; 2) assembly of characterization equipment, including a microfluidic fluid handling system capable of ultra-low fluid flow rates, a Zeiss microscope, and an Ocean Optics spectrometer; and 3) design and testing of an amperometric electrochemical detector for fatty acids that can be integrated with the output of the nanofluidic chromatographic channel.

B. PROGRESS AND RESULTS

Task 1: In conventional size exclusion chromatography (SEC), molecules are separated based on their transit time through a size exclusion network formed by closely packed small (~10μm) silica or polymer beads with uniform nanopores. We have designed a nanofluidic size exclusion chromatograph (nSEC) consisting of a column containing size-exclusion gaps defined in the z-direction, analogous to SEC's nanopores, over a matrix of microchannels in the x-y plane, similar to the interstices between the beads in traditional SEC (Fig. 1). Several geometrical variations of this design have been fabricated in order to determine the optimal channel separation geometry. We have developed a model for the nSEC column which predicts that the analyte will be efficiently separated based on molecular size by differential diffusion into the planar size-exclusion gap. Figure 2 shows a modeled chromatogram, similar to that obtained by conventional SEC, of the separation of an analyte mixture of nanobeads in the nSEC.

Our device fabrication process has evolved from the original strategy based on the nanochannel fabrication technologies of Harold Craighead's group at Cornell University [1] in order to overcome several fabrication challenges. Optimization of each etching step and its associated pattern definition and removal processes, including both photolithography and metalization, has required multiple runs for fine adjustment of several operating parameters specific to each process step and material in order to obtain a robust, reproducible technology. We have successfully ion milled 100nm tall support posts (for definition of the size exclusion gap) in silicon, and used reactive ion etching to create the channel matrix (Fig. 3). The following fabrication steps resulting in a sealed device are on schedule to be completed by the end of the FY02 award: growth of a ~1-2nm thermal oxide layer over the entire wafer to create a chemically uniform surface (critical for eliminating analyte/surface interactions), water-jet drilling of interface ports, and anodically bonding a cover glass to form the device roof.

Task 2: Essential to the success of obtaining interpretable qualitative and quantitative data from chromatographic separations is the ability to generate and precisely control ultra-low fluid flow. We have designed and are implementing a custom fluid-handling system (Fig. 4) capable of continuous reliable ultra-low fluid flow rates. Initial fluidic tests are scheduled to begin under the FY02 award. For characterization of the fluidic flow through the nSEC, the appropriate fluorescent microscopy components have been assembled in order to detect a series of different-sized fluorescently labeled nanospheres, which are to be flown through the column.

Task 3: Prof. Wang and his group at NMSU have developed several electrochemical detection methodologies suitable for detection of many biological molecules. A novel contactless conductivity technique using aluminum film electrodes deposited on the outside of a PMMA fluidic channel detected ions in a solution at μM concentrations (Fig. 5). The response was reproducible and linear over a broad range (20μM – 7mM) at an operating voltage of 5 V. Additionally, NMSU has developed techniques for amperometric detection of amino acids, peptides, and organic peroxides, which in themselves are relevant detection capabilities, but also provide groundwork for detecting other nonelectroactive analytes, such as fatty acids. Current efforts focus on labelling techniques involved for fatty acid detection in connection with the fundamental benchtop chemistry for amperometric detection. The necessary combination of redox markers and electrode materials is on schedule to be determined under the FY02 award.

C. SIGNIFICANCE OF RESULTS AND EXPECTED RESULTS

The novel contactless conductivity measurement demonstrated by NMSU obviates many problems (e.g. fouling, unwanted reactions) associated with the electrode-solution contact, offers isolation of the detection system from high separation fields (used in capillary electrophoresis), does not compromise the separation efficiency, and greatly simplifies the detector fabrication. With further optimization to lower detection limits, the contactless approach will enhance the power and scope of microfluidic chemical analyzers.

Few experimental studies have been conducted to study fluid flow in the nanoregime, and to our knowledge no one has developed a microscale size exclusion chromatograph. Successful separations in the nSEC determined by the size exclusion gap, which is expected to range from ~10 to 100nm, offers the opportunity to study the effects of geometry, fluid velocity, and surface interactions on the nanoscale. Our ability to directly manipulate and control nanoscale features in the separation column should allow significantly greater control of the size exclusion process compared to conventional SEC columns. It may also result in the separation of a greater number of bands with narrower individual bands than can be achieved with conventional SEC.

If successful, the nSEC could eventually be used to detect of a wide variety of organic macromolecules and non-polar molecules that cannot be separated by capillary electrophoresis or other microfluidic/miniature separation methods. Our particular development effort is focused on optimizing the nSEC for the analysis of lipid compounds and their breakdown products including fatty acids, high-molecular-weight lipid polymers, and crude oils. Subsurface oils, for which conventional SEC is a well-established standard terrestrial technique, and fatty acids, to which successful SEC analysis has been applied [2], are of particular astrobiological interest. Fatty acids are the building blocks for lipid bilayer membranes found in all terrestrial cellular organisms, are extremely stable over geological time scales, and are not expected to decompose under the conditions found in subsurface soil or ice. Biologically-derived fatty acids on Earth can be determined by their preference for an even number of carbon atoms with concentrations peaking around 14 to 22 atoms. In contrast, nonbiological fatty acid samples potentially found on other planetary bodies are not expected to show a similar preference or pattern. On Earth, the largest identifiable trace of past life is generally thought to be subsurface oil deposits [3]. Similarities between the Earth and Mars at the time of formation suggest large deposits of oil beneath the Martian surface. Due to the lack of plate tectonic signatures, less volcanic activity, unlikely burial of ancient rocks by sedimentation, and lower subsurface temperatures and pressures, hydrocarbon deposits formed on Mars billions of years ago are likely to have survived near the surface. Subsurface oil on Mars would thus be an excellent place to look for evidence of past life and perhaps for extant life, as oil would provide unoxidized carbon fuel.

The nSEC system with electrochemical detection is suitable for potential *in situ* instrumentation for planetary missions as it is expected to have a minimum number of components, perform a minimum number of discrete operations, be robust and capable of withstanding space environments, and have low mass and low power requirements.

D. FINANCIAL STATUS

The total funding for this task was $320,000, of which $250,000 has been expended.

E. PERSONNEL

Partha Shakkottai (353) has been instrumental in modeling the nSEC column. Linda del Castillo (345) and Victor White (384) have fabricated the nSEC devices. Martin Pumera (NMSU) has had a major role in development of the electrochemical detection techniques.

F. PUBLICATIONS

[1] M. Pumera, J. Wang, F. Opekar, I. Jelinek, J. Feldman, H. Löwe, and S. Hardt, "A Contactless Conductivity Detector for Microchip Capillary Electrophoresis," *Anal. Chem.,* 74, 1968 (2002).

[2] J. Wang, A. Escarpa, M. Pumera, J. Feldman, "Capillary Electrophoresis-Electrochemistry Microfluidic System for the Determination of Organic Peroxides," *J. Chromatogr. A*, 952, 249 (2002).

[3] D. Svehla, S. Feldman, J. Feldman, F. Grunthaner, P. Shakkottai, L. del Castillo, and V. White, "Nano-fabricated Size Exclusion Chromatograph," *Proc μTAS 2002 Symp.*, in press (2002).

[4] J. Wang, G. Chen, and M. Pumera, "Microchip Separation and Electrochemical Detection of Amino Acids Following Precolumn Derivatization with Naphthalene-2,3-dicarboxyaldehyde," *Electroanalysis*, in press (2003).

[5] J. Wang, and M. Pumera, "Nonaqueous Microchip Capillary Electrophoresis," submitted.

G. REFERENCES

[1] S.W. Turner, A.M. Perez, A. Lopez, and H.G. Craighead, "Monolithic Nanofluid Sieving Structures for DNA Manipulation" *J. Vac. Sci. Technol. B*, 16, 3835 (1998).

[2] C.M. Dobarganes, and G. Marquez-Ruiz, *Advances in Lipid Methodology Two* (ed. W.W. Christie), Dundee: Oily Press, 1993, pp. 113-137.

[3] J.F. McGowan, "Oil and Natural Gas on Mars," *Proceedings of SPIE-Instruments, Methods, and Missions for Astrobiology III*, 4137, 63 (2000).

H. APPENDIX: *FIGURES*

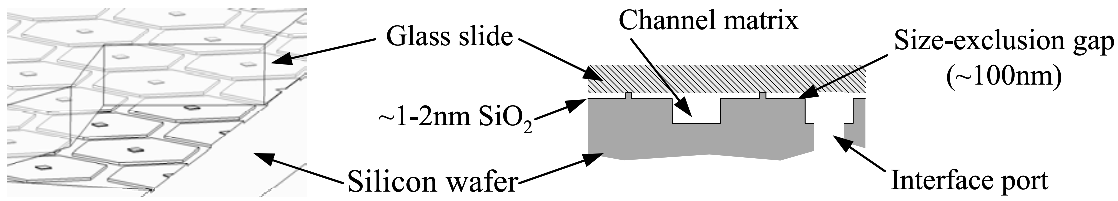

Figure 1: 3-D and cross-sectional views of nSEC schematic

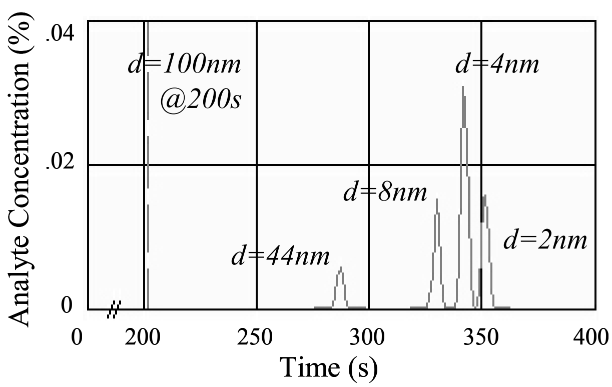

Figure 2: Chromatogram of modeled nSEC separation

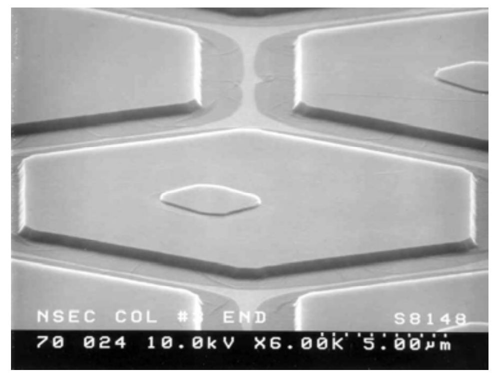

Figure 3: nSEC channel matrix and 100nm tall support posts

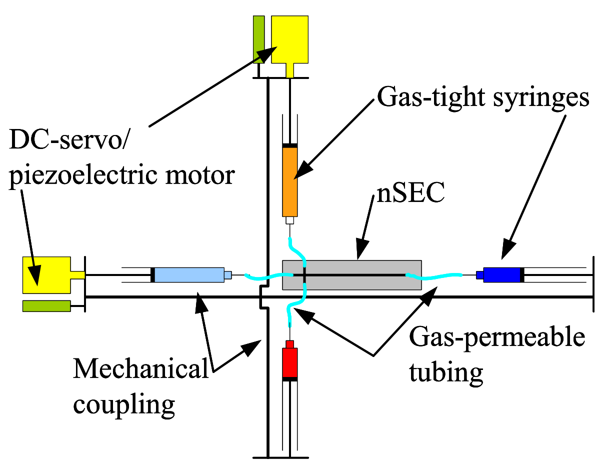

Figure 4: Schematic of continuous ultra-low fluid flow (sub-pL/s – µL/s) pump system: two sets of mechanically coupled syringes driven by a closed-loop DC-servo/piezo stack actuator. Gas in the system is evacuated via the gas-permeable tubing.

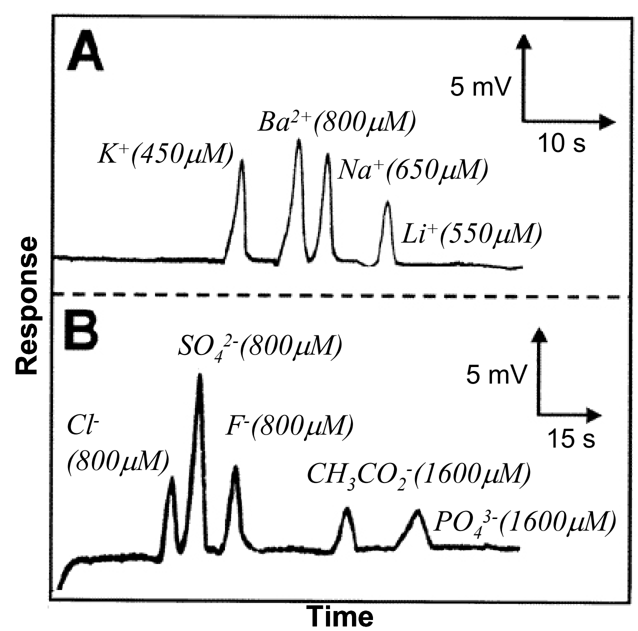

Figure 5: Electorgrams of cation (A) and anion (B) solutions using contactless conductivity detection for capillary electrophoresis separations.